In Vitro Evaluation of Wound Healing by Phenytoin 2% and Misoprostol 0.0024% Topical Hydrogel (EctoSeal P2G) and Poloxamer Gel

Introduction & Methodology:

Re-epithelialization is a process in wound healing that involves the migration of keratinocytes (cells within the epidermal layer of the skin) from the edge, towards the center of the wound, to form a thin layer of cells over the exposed area. The rate at which keratinocyte migration occurs is important in wound healing as it is the body's first attempt at restoring the protective skin layer. Delays in this healing process may result in wound infections and hypertrophic skin scarring. The purpose of this study was to assess the ability of the test formulations Phenytoin 2% and Misoprostol 0.0024% Topical Hydrogel (EctoSeal P2G) (PCCA Formula 14811), and Phenytoin 2% and Misoprostol 0.0024% in Poloxamer Gel, to facilitate keratinocyte migration by evaluating *in vitro* the process of re-epithelialization, using primary human keratinocytes.

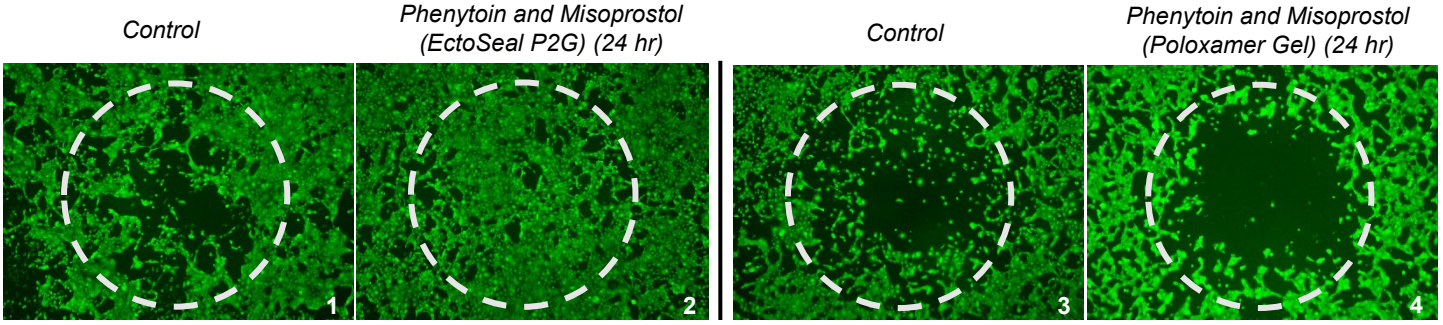
The *in vitro* evaluation was performed using the Oris™ cell migration assay kit (Platypus Technologies, Inc.), which consists of a 96-well plate, cell seeding stoppers that inhibit the spread of cells into the migration zone (center of the wells), and a black mask that allows for detection of cell migration. The cells were treated with the test formulations for 24 hours and then stained with Calcein AM, a non-fluorescent dye that is converted to green fluorescent calcein by viable cells. Fluorescence was detected using the CLARIOstar® plate reader (BMG Labtech) with Stars software for analysis at 483/14 excitation and 530/30 emission wavelength.

Results and Discussion:

The abilities of the test formulations to facilitate migration of primary human keratinocytes into the migration zone was quantified based on the green fluorescence detected by the plate reader, which is expressed in terms of Relative Fluorescence Unit (RFU). Phenytoin 2% and Misoprostol 0.0024% Topical Hydrogel (EctoSeal P2G) at 24 hours showed a mean change of 70.62% from control, which means that the topical hydrogel significantly promoted cell migration. In the contrary, Phenytoin and Misoprostol in Poloxamer Gel did not increase cell migration (negative mean change) (Table 1 and Figures 1-4). Keratinocyte migration is part of the re-epithelialization process in wound healing and, therefore, the EctoSeal formula is likely to have greater wound healing abilities than the corresponding formula for Poloxamer Gel.

Table 1. *Mean Relative Fluorescence Units (RFU) ± SD and mean change (%) ± SD for Phenytoin and Misoprostol (EctoSeal P2G), and Phenytoin and Misoprostol (Poloxamer Gel), from control following 24 hours post-application.*

Test formulations	Mean RFU ± SD	Mean change (%) ± SD	*p*-value
Phenytoin and Misoprostol (EctoSeal P2G)	121,381 ± 30,784	70.62 ± 43.27	6.95E-06
Phenytoin and Misoprostol (Poloxamer Gel)	87,362 ± 28,507	-29.99 ± 22.85	0.0007



Figures 1-4. *Keratinocyte migration (green fluorescence) for Phenytoin and Misoprostol (EctoSeal P2G) (1-2), and Phenytoin and Misoprostol (Poloxamer Gel) (3-4), from control following 24 hours post-application.*